CHEMICAL COMPOSITION OF THE GREEN ALGA *BOTRYOCOCCUS BRAUNII*

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ABSTRACT — The growth and chemical composition of the green alga *Botryococcus braunii* grown in a modified and improved basal medium under batch conditions have been examined and compared with composition of the protein-rich filamentous cyanobacterium *Spirulina platensis*. The growth rate and biomass yield of the alga was enhanced to two fold in the modified medium while the profiles of amino acids and lipids (saturated and unsaturated fatty acids) remained almost unaffected. The results are discussed with respect to carbohydrate, protein, amino acids, lipid and fatty acid composition of *Botryococcus braunii* in improved medium in relation to its economic importance.

RÉSUMÉ — La croissance et la composition chimique de l'algue verte *Botryococcus braunii*, cultivée en batch dans un milieu de base modifié et amélioré, ont été examinées. Sa composition chimique a été comparée à celle de la cyanobactérie filamenteuse riche en protéines *Spirulina platensis*. Le taux de croissance et le rendement en biomasse de *B. braunii* est deux fois meilleur dans le milieu modifié, tandis que les profils en acides aminés et en lipides (acides gras saturés et insaturés) restent presque inchangés. Les résultats concernant la composition en hydrates de carbone, protéines, acides aminés, lipides et acides gras de *B. braunii* dans le milieu amélioré sont discutés au regard de l'importance économique de cette algue. (Traduit par la Rédaction)

KEY WORDS: *Botryococcus braunii*, Chlorophyta, culture medium, chemical composition, Cyanophyta, cyanobacterium, microalga, *Spirulina platensis*.

INTRODUCTION

The production of photosynthetic biomass via reduction of atmospheric CO_2, is a promising source of food and energy, since it is renewable. In aquatic ecosystems, algae are the major biomass producers, and for these reasons, considerable attention has been paid to the exploitation of the potentials of micro algae as food, feed and fuel (Shelef & Soeder, 1980; Becker, 1993). Amongst the various species, the filamentous cyanobacterium *Spirulina platensis* (Nordstedt) Geitler is an attractive source of single cell protein of a high nutritive value (Ciferri, 1983). However, the major disadvantage is the requirement of high nitrate nitrogen and alkaline medium for growth (Zarrouk, 1966). The green colonial alga *Botryococcus braunii* Kützing occupies a unique position in being a rich source for production of hydrocarbons, and total lipids. It also is supposed to be ancestral for the origin of Boghead coals and natural rubbery deposit, the coorongite (Largeau *et al.*, 1980; Chirac *et al.*, 1985). Vegetative cells of *B. braunii* may accumulate unusually high levels of lipids and hydrocarbon rich lipids, amounting to 30-70% of its dry weight under different conditions of growth (Wolf, 1983; Yamaguchi *et al.*, 1987). This high production of hydrocarbons seems to influence the growth and total biomass productivity adversely (Belcher, 1968; Casadevall *et al.*, 1985). Therefore, numerous attempts have been made in the past to develop suitable media for growing *B. braunii* under alternative conditions for sustained production of algal biomass with higher quantities of hydrocarbons (Sawayama *et al.*, 1992, 1994). In this communication we report the growth of the alga in modified, improved, basal medium under batch conditions and compare the chemical composition, fatty and amino acid profiles of *Botryococcus braunii* with those of *Spirulina platensis*.

MATERIALS AND METHODS

The colonial green alga *Botryococcus braunii* UTEX 572 and the filamentous blue green-alga (cyanobacterium) *Spirulina platensis* were obtained from Austin culture collection, USA and from Prof. Riplay D. Fox, France, respectively. *S. platensis* was grown in a modified Zarrouk's medium (1966) while *Botryococcus braunii* was grown in a modified, improved, medium that of Chu No. 10 (Safferman & Morris, 1964) having the following composition of macro elements KNO_3 (0.4 g l^{-1}), $MgSO_4$ $7H_2O$ (0.05 g l^{-1}), K_2HPO_4 (0.05 g l^{-1}), Na_2CO_3 (0.04 g l^{-1}), $CaCl_2$ $2H_2O$ (0.075 g l^{-1}), $C_6H_5O_7$ Fe $5H_2O$ (0.0035 g l^{-1}), Na_2 EDTA (0.0035 g l^{-1}) and 1 ml of micro element solution per litre was added containing H_3BO_3 (2.86 g l^{-1}), $MnCl_2$ $4H_2O$ (1.8 g l^{-1}), $ZnSO_4$ $7H_2O$ (0.22 g l^{-1}), $CuSO_4$ $5H_2O$ (0.079 g l^{-1}) $CoCl_2$ $6H_2O$ (0.04 g l^{-1}), Na_2MoO_4 $2H_2O$ (0.025 g l^{-1}) and medium was also supplemented with 5% (v/v) soil extract (Rai *et al.*, 1987). Algal cultures were gown in a culture room maintained at 24 ± 1° C and illuminated at an intensity of 40 µmol m^{-2} s^{-1} (16 h/8 h light/dark cycle).

The growth of the algae was determined via dry weight measurements and total chlorophyll content extracted in methanol according to MacKinney (1941). For analysis of chemical constituents, the algal samples were harvested, repeatedly washed to remove salts and dried in oven at 60° C for 6-8 h before further use. The total protein content of the algae was determined by folin-phenol reagent (Lowry *et al.*, 1951), carbohydrate content by phenol-sulphuric acid reaction (Dubois *et al.*, 1956) and lipids by acid dichromate method (Amenta, 1964). The hydrocarbons in the algal cells of stationary phase were extracted from the freeze-dried cells by sonication (MSE Soniprep 150) with hexane and estimated according to Maxwell *et al.* (1968). To analyse amino acid composition, aliquots of 25 mg of dried, defatted and powdered samples were hydrolysed with 6 N HCl, flushed with nitrogen, for 24 h at 104-110° C. The hydrolysate was concentrated to dryness under vacuum and the residue was dissolved in 66 mM sodium citrate buffer (pH 2.2) and analysed on a LKB 4101 Amino acid analyser for amino acid profile. For fatty acid analysis, aliquots of the algal samples were saponified, methylated according to Cocks & Rede (1966) and analysed by gas chromatography as described earlier (Prakash & Pal, 1992). The data presented are average of three replicates of three independent experiments conducted under identical conditions.

RESULTS AND DISCUSSION

The growth and chemical composition of *Botryococcus braunii* obtained in the basal and improved media in batch cultures are compared in Table 1. In the improved medium, calcium nitrate, sodium silicate and citric acid of the basal medium (Chu No. 10) were replaced with potassium nitrate, calcium chloride, sodium EDTA and soil extract. The final yield of total biomass obtained in the modified, improved, medium was 46% higher when compared to the basal medium. In the improved, modified, medium the generation time of the alga was reduced to half, and specific growth rate was increased over the values obtained in the basal medium. Chemical analysis showed relatively higher amounts of total protein (22-24%), lipids (44-46%) and chlorophyll (13.98 mg l^{-1}) in the modified medium while carbohydrate level did not change significantly. The hexane fractions of the algal samples grown in basal and improved medium showed that hydrocarbons account for about 12 ± 1% and 17 + 1% of total lipids respectively. Our data have confirmed previous results that in the improved medium, *Botryococcus braunii* is able to produce and accumulate large amount of lipids in the stationary phase of batch cultures (Ben-Amotz *et al.*, 1985; Fogg, 1988).

Table 1. Growth and chemical composition (dry wt %) of *Botryococcus braunii* grown in different media after 20 days of incubation.

	Basal medium	Improved medium + soil extract (5% v/v)	% increase in modified medium
Dry weight (g l^{-1})	0.78 – 0.82	1.14 – 1.18	46.0
Total chlorophyll (mg l^{-1})	10.90 – 11.00	13.98 – 14.25	28.0
Specific growth rate (μm h^{-1})	5.3 10^{-2}	10.2 10^{-2}	117.0
Doubling time (h)	130 – 132	68 – 70	91.0
Protein (%)	18 – 20	22 – 24	21.0
Carbohydrates (%)	10 – 13	12 – 14	12.5
Lipids (%)	40 – 42	44 – 46	10.5
Hydrocarbon* (% of total lipids)	11 - 13	16 – 18	42.0

* Hydrocarbons were extracted from 30 days old culture. All the values are mean of three replicates of three independent experiments

The amino acid composition of *Botryococcus braunii* grown in the basal and the improved medium, along with *Spirulina platensis* and FAO standard (1973), are compared in Table 2. The levels of essential amino acids like threonine, valine methionine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine were relatively higher for *Botryococcus braunii* grown in the modified and improved medium compared to the basal medium. Despite the increase in protein content of the alga grown in the modified, improved, medium, the amino acid profile of the alga did not show large changes over the control except for arginine which was higher in the algal samples grown on the improved medium.

Table 2. Amino acid composition (g/16 g N) of *Botryococcus braunii* grown in different media and of *Spirulina platensis*.

Amino acids	*B. braunii* BM	*B. braunii* IM	*S. platensis* (Zarrouk's medium)	FAO Standard (1973)
Essential				
Thr	5.6	5.9	5.8	4.0
Val	6.2	6.5	8.8	5.0
Met	0.5	0.7	2.1	3.5
Ile	4.5	4.8	7.6	4.0
Leu	7.4	7.9	7.2	7.0
Phe	5.7	5.9	4.4	
Lys	3.7	3.9	5.0	5.5
His	1.2	1.4	1.9	
Arg	4.1	6.0	5.8	
Non essential				
Asp	10.1	9.9	8.4	
Ala	8.2	7.6	7.5	
Ser	4.5	4.4	5.6	
Glu	12.5	12.9	8.2	
Pro	5.1	5.1	5.4	
Gly	8.7	8.2	8.1	
Cys	1.0	0.8	0.4	3.5
Tyr	2.6	2.6	2.6	6.1

BM = Basal medium; IM = Improved medium + soil extract (5 % v/v).

Composition of some of the essential amino acids in *Botryococcus braunii* grown on the improved medium were similar to those of *Spirulina platensis* and the FAO standard for essential amino acids in plant proteins. The levels of most of the essential amino acids were quite balanced except for methionine, histidine and lysine.

Table 3 presents the comparison of fatty acid composition of *Botryococcus braunii* and *Spirulina platensis*. Amongst the saturated fatty acids, lauric (12:0), myristic (14:0) and stearic (18:0) acids were in low amounts while palmitic (16:0) was the major saturated fatty acid in both the algae although its percentage was about 2-fold higher in *S. platensis*. Oleic (18:1), linoleic (18:2) and linolenic (18:3) were the major unsaturated fatty acids in *Botryococcus braunii*. The level of oleic acid was 5.7-fold higher in *Botryococcus braunii* and the level of linoleic acid was 2.6 -fold higher in *Spirulina platensis*, while the level of linolenic acid was nearly the same in both the algae. The remaining fatty acids in *Botryococcus braunii* also showed marked differences with *Spirulina platensis*. The amount of total unsaturated fatty acids in *Botryococcus braunii* was about 2 — fold higher than in *Spirulina platensis*. The saturated to unsaturated fatty acid ratio and total level of unsaturated fatty acids showed that lipid quality in *Botryococcus braunii* was superior to that of *Spirulina platensis*, whereas no marked differences were observed in the fatty acid composition of *Botryococcus braunii* grown in the basal or improve media. These results on fatty acid profile in *Botryococcus braunii* are in close agreement with earlier reports by Douglas *et al.* (1969), Dubinsky *et al* (1978) and Ben-Amotz *et al.* (1985).

Cyanobacteria and green plants (including eukaryotic green algae) differ in lipid composition and in the biosynthetic pathway of lipids. The former contains monogluco-

Table 3: Fatty acid composition of *Botryococcus braunii* and *Spirulina platensis*.

Fatty acid	% of total methylated fatty acid mixture		
	B. braunii		*S. platensis*
	BM	IM	(Zarrouk's medium)
Lauric acid (12:0)*	2.1	2.4	0.7
Myristic acid (14:0)	2.6	2.8	1.1
Tetradec-5-enoic acid (14:1)	0.4	0.3	0.6
Palmitic acid (16:0)	18.1	17.8	35.2
Hexadec-9-enoic acid (16:1)	1.3	1.2	8.5
Stearic acid (18:0)	1.6	1.7	0.6
Oleic acid (18:1)	38.3	38.6	6.8
Linoleic acid (18:2)	8.6	8.3	21.2
Linolenic acid (18:3)	20.8	20.6	19.8
Unidentified	6.2	6.3	5.5
Total unsaturated	69.4	69.0	37.6
Saturated : Unsaturated	0.35	0.35	0.66

* Number of carbon: number of double bonds; BM = Basal medium; IM = Improved medium + soil extract (5 % v/v); unsat- unsaturated; sat- saturated.

syl, diacylglycerols which are synthesized by transfer of glucose unit from UDP-glucose to diacylglycerol (Sato &Murata, 1982). By contrast, green plants contain phosphatidylcholine directly synthesized by transfer of the galactose unit from UDP-galactose to diacylglycerol (Roughan & Slack, 1982). However, in *Botryococcus braunii* the interesting fact is the shift and diversion of photosynthetic driven reductant into the efficient synthesis and accumulation of saturated and unsaturated fatty acids and hydrocarbons. The slow growth rate of the alga has been attributed to changes in cellular structure, membrane fluidity and shift in physiological metabolism due to the accumulation of long-chain hydrocarbons botryococcenes (Wolf *et al.*, 1985; Fogg, 1988). By contrast, cyanobacteria have a very simple lipid composition, similar to the chloroplast of higher plants , which plays a central role in regulating membrane fluidity during thermoadaptation and growth (Golecki & Drews, 1982). This reflects a different composition and mechanism of lipid metabolism in *Botryococcus braunii* and *Spirulina platensis*. Therefore, in view of our studies, the colonial green alga *Botryococcus braunii* seems to offer an economically viable system for utilizing solar energy in the production of hydrocarbons due to its ability to accumulate a high lipid content with balanced amino acid profile and enhanced growth rate in the improved basal medium (Ben-Amotz *et al.*, 1985; Wolf *et al.*, 1985).

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